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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Immunotherapy is rapidly becoming accepted as an effective way to treat human cancer. A large proportion of B-cell malignancies express the product of their re-arranged immunoglobulin (IG) genes on the cell surface. Surface-expressed IG constitutes a de facto tumor-specific target antigen. Here we report that two B-cell tumor cell lines, JVM13 and Mec1, express surface IG. Therefore we cloned and sequenced the cDNA encoding the variable portions of these IGs. Specifically, we identified the CDR3 sequences of the heavy and light chain variable portions for each IG, which constitute the molecular target. We have now engineered the following CDR3 sequences "ARSQGVLTAIDY"/"QQYYSIPYT" for the Mec1 heavy and light chains respectively and "ASSYYDILTGYLYYYFDY "/"SSYTSSSTLMI" for the JVM13 heavy and light chains, into a model antibody 4D5 (see figures 1-5 in the report). The "Tomlinson" human antibody phage library will be used to pan for antibodies that bind these target CDR3s and not the parent 4D5 antibody. To confirm the utility of the Tomlinson library and gain experience with handling this complex selection system we have confirmed selection of phage to a test antigen. Successful expression will lead directly to the selection of CDR3-specific antibody-encoding phage: from which we will make our final immunotherapeutic agents.

15. SUBJECT TERMS
Immunotherapy, B-Cell tumor, cancer

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1. **INTRODUCTION:**

In the US the incidence of newly diagnosed B-cell leukemias and lymphomas amounts to approximately 100,000 cases per year, leading to 40,000 deaths in the same population. While current treatments are improving, side effects from traditional chemotherapy can be debilitating often causing significant life-long morbidity. Compared with chemotherapy, immunotherapy promises similar or better results with far fewer side effects. A potential treatment for individuals who display immunoglobulin protein on the surface of their malignant cells, is targeted immunotherapy that is cytotoxic for only tumor cells. But such immunotherapy strategies are usually time-consuming and complex to organize. To address this limitation we are attempting to speed and simplify the process of producing antibodies to tumor- expressed surface immunoglobulin. Additionally, once we produce anti-tumor antibodies we plan on 'arming' them with toxins. In this way we will generate novel immunotherapeutic treatments for B-cell malignancies.

2. **KEYWORDS:**

immunoglobulin, lymphoma, leukemia, immunotherapy phage display, toxin,
single chain Fv.

3. OVERALL PROJECT SUMMARY:

Revised Statement of work.

As of July 2014 we completed all parts of Tasks 1'3. Task 3 was completed since the last annual report so details of its successful completion are reported below. This leaves Tasks 4 and 5 in need of completion with a new schedule for completion.

Task 4. Convert single chain Fv from library into a therapeutic antibody
4A.Convert single chain Fv into immunotoxin D end of August 2014
4B. Convert single chain Fv into a full length antibody D end of August 2014
4C. Convert single chain Fv into a Chimeric antigen receptorD end of August 2014.
Notes: converting single chain Fv from selected phage is a simple matter of PCR cloning into existing vectors. Vectors for 'immunotoxin', 'full length antibody' and 'chimeric antigen receptor' are all ready to receive the cloned single Fv sequences. This is why we can aim for the same completion date of Aug31st for each of these subDtasks.

Task 5. Conduct preliminary experiments in tissue culture to characterize the cytotoxic activity of candidate immunotoxins, full length antibodies and chimeric antigen receptor constructs D Sept and Dec 2014. Because our cloning step is several months behind, the tissue culture experiments anticipated in Task 5 are pushed back and now will be performed between Sept and Dec 2014.

Report on the completion of Task 3.

Task 3. Selection of phage that bind to the purified candidate antibody and not to irrelevant antibodies. Phage are from single chain Fv library. Sequence phage that bind to candidate antibodies.

3A. Select binders from phage library D month 9.
3B. Enrich for high affinity binders via multiple rounds of selection D month 10D11.
3C. Sequence phage at the end of three rounds of panning and verify enrichment and sequence as a single chain Fv D month 12D13.

Report:
Previously, we reported on the cloning and sequencing of the rearranged immunoglobulin genes from the MEC-1 and JVM-13 human lymphoma cell lines (Tasks 1 and 2).

Task 3.
The CDR3 sequences from the MEC-1 and JVM-13 Ig genes were grafted into the scaffold single chain Fv antibody, called 4D5 Figs 1 and 2. Both heavy and light chain sequences were grafted into the corresponding locations within the single chain Fv of 4D5, replacing the endogenous CDR3 sequences. We made chimeric scFv constructs with FLAG-tags at the N-terminus and from these plasmids we expressed novel recombinant target proteins. Fig 1 shows a schematic depicting the structure of 4D5 and 4D5 with MEC-1 CDR3 sequences. Fig 2 depicts the structure of 4D5 and 4D5 with JVM13 CDR3 sequences. Thus 4D5-Mec and 4D5-JVM became 'targets' for phage selection using the Tomlinson I antibody library. In summary, we were seeking phage that bound to either 4D5-MEC or 4D5-JVM but did not bind to the parent 4D5 antibody. In this reporting cycle, we are happy to summarize substantial completion of Task 3.

Fig 3 shows a schematic of an M13 phage displaying a single chain Fv. The Tomlinson I phage library contains approximately 1.4 x 10^8 distinct scFvs of unique specificity, each scFv displayed in the location shown in Fig 3. When we panned this phage library on the 4D5-MEC protein we enriched for 5 phage that bound 4D5-MEC but not the 4D5 parent - see Fig 4. The scFv DNA of each of these phage was then sequenced and found to be unique. The DNA sequences are provided in Figs 5-9 and a multiple alignment is shown in Fig 10. We had trouble refolding the 4D5-JVM protein and noticed that it had a tendency to aggregate. No phage were isolated that exhibited specific binding to 4D5-JVM. Because this represented a technical hurdle and we had good results isolating binders to 4D5-MEC, we advanced the project characterizing the phage that bound to this protein. Fig 11 shows a FACS analysis of phage binding to the surface of Mec-1 cells. This result represents a remarkable achievement. Apparently, the 4D5-Mec recombinant protein looks sufficiently like the surface of the actual MEC-1 cells that binding is retained by all the reactive phage.

As mentioned above, despite extensive panning there were no binders isolated from the Tomlinson I library to 4D5-JVM target protein. We also evaluated the Tomlinson J library extensively and likewise failed to enrich for phage that bound specifically to 4D5-JVM. The reasons for obtaining 5 binding phage to 4D5-MEC and none to 4D5-JVM are currently not understood but are likely related to poor folding of the 4D5-JVM protein. Thus, going forward we are focusing our efforts on the five selected phage, labeled 1, 5, 7, 9 and 10 that bound the 4D5-MEC antibody and also bound the MEC-1 cells. Upon sequencing 2 of the 5 phage we noted stop codons in the framework regions - see Figs 6 and 9. Because phage are grown in tRNA 'TAG-suppressor' strains of E coli, read-through was

allowed and antibody Fv sequences were displayed. However, for expression in mammalian cells or in normal E coli strains, these sequences had to be corrected. Stop codons in suppressor strains are normally read as glutamic acid. This posed a problem going forward. We could replace the stop sequences with a codon encoding glutamic acid and expect to get the same amino acid as inserted by the suppressor strain or we could replace the codon with one that re-introduced the natural framework residue. For Tasks 4 and 5 we have chosen to do both.

4. **KEY RESEARCH ACCOMPLISHMENTS:**

- We have produced single chain Fv proteins with engrafted sequences taken from the CDR3 sequences of surface immunoglobulins on B-cell tumors (see Fig 1 right hand panel). Producing such single chain Fv proteins, per se, does not constitute the Key Accomplishment. Rather the Key Accomplishment is noted because phage that bind the 4D5-MEC protein **ALSO** bind the surface of MEC-1 cells. Thus, these single chain Fv proteins mimic a structure on the surface of tumor cells. This was the major conceptual goal of the project.

5. **CONCLUSION:**
It is possible to use cloned sequences from human tumor cells to design mimics of surface immunoglobulin on B-cell tumors. This will improve our ability to produce antibody-based therapeutics.

6. PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS:
Nothing to report.

7. **INVENTIONS, PATENTS AND LICENSES:**
Nothing to report.

8. **REPORTABLE OUTCOMES:**
Phage display was used to select single chain Fvs that bind CDR3 sequences from B-cell tumors. Five phage were isolated, each one capable of binding the surface of the target B-cell.

9. **OTHER ACHIEVEMENTS:**
Nothing to report.

10. **REFERENCES:** List all references pertinent to the report using a standard journal format (i.e., format used in *Science*, *Military Medicine*, etc.)
Nothing to report.

11. **APPENDICES:** Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

Pages shall be consecutively numbered throughout the report. DO NOT RENUMBER PAGES IN THE APPENDICES.

NOTE:

TRAINING OR FELLOWSHIP AWARDS:
Not applicable.

COLLABORATIVE AWARDS:
Not applicable.

QUAD CHARTS:
Not applicable.

DELINQUENT REPORTS
Not applicable.

MANUSCRIPTS/REPRINTS
Not applicable.

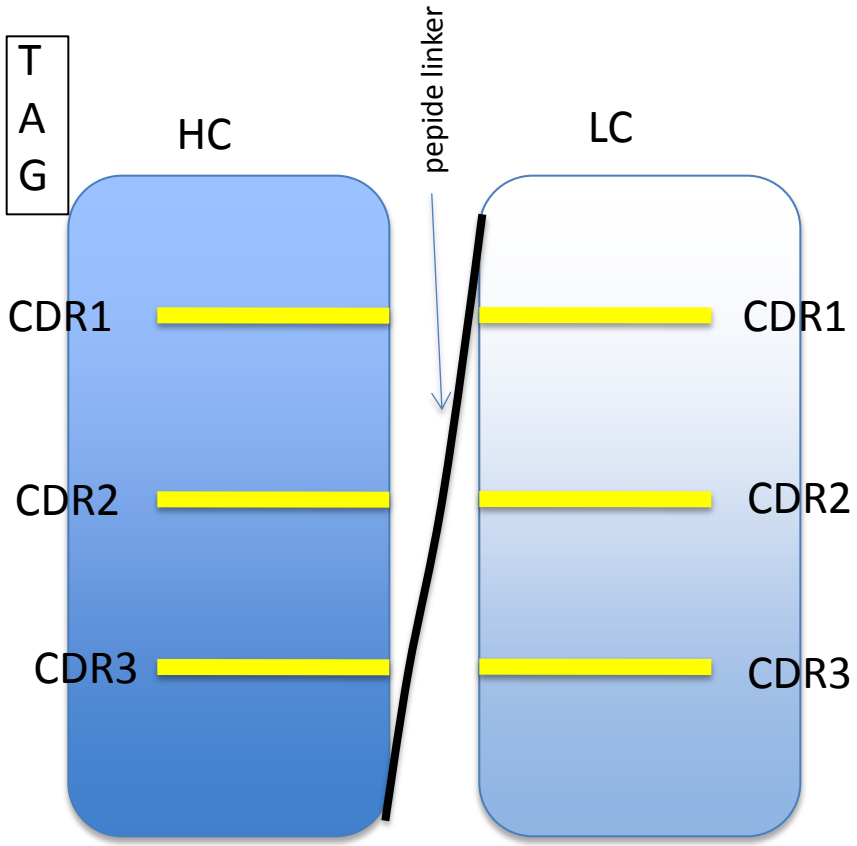
ABSTRACTS
Not applicable.

MARKING OF PROPRIETARY INFORMATION:
Not applicable.

Figures:

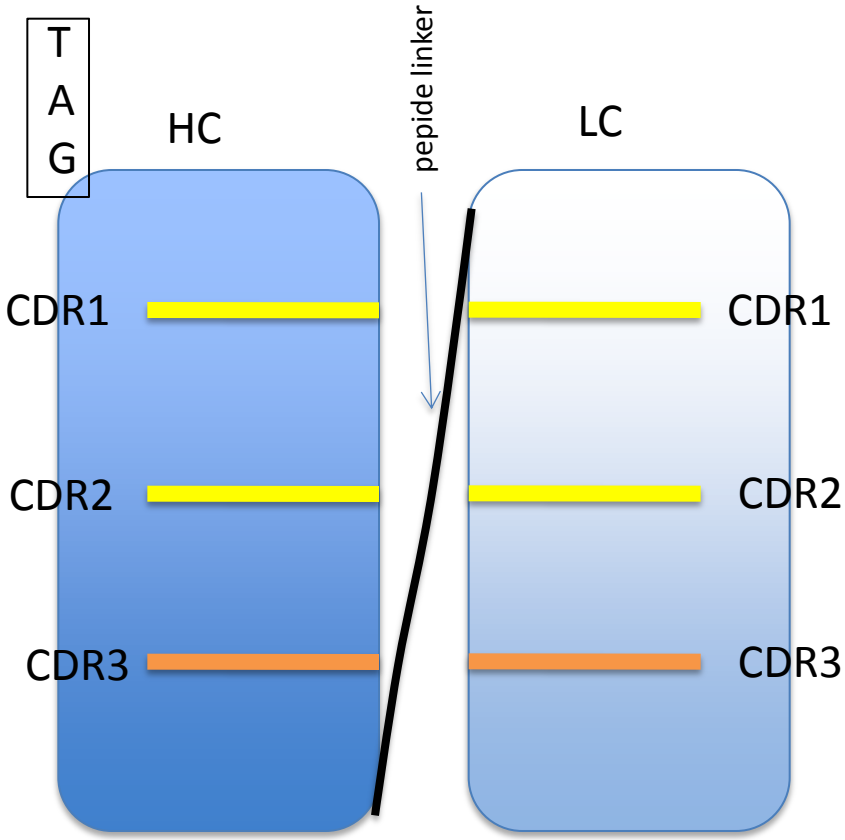
Fig 1

A. hum4D5 scFV - WT



H-CDR3 = SRWGGDGFYAMDY 13aa
L-CDR3 = QQHYTTPPT 9 aa

B. hum4D5 scFV – Mec1_CDR3s



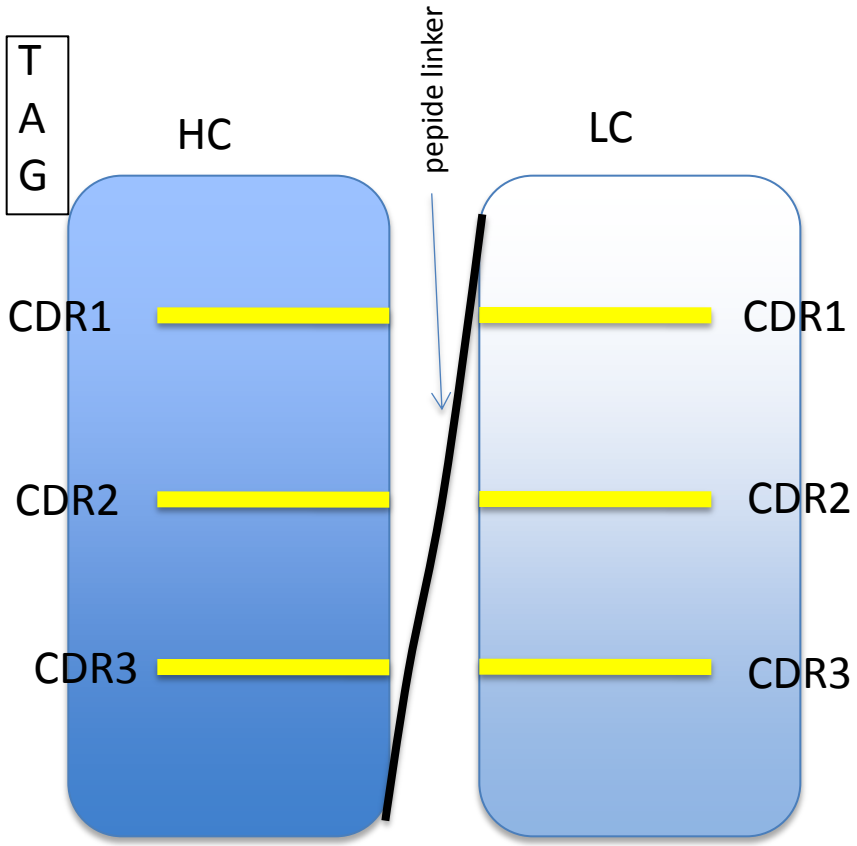
H-CDR3 = ARSQGVLTAIDY 12aa
L-CDR3 = QQYYSIPYT 9aa

Legend to Fig 1.

Generation of target proteins by inserting MEC1 CDR3 sequences into the scaffold antibody, 4D5. The idealized structure of the single chain Fv of the 4D5 antibody is shown. The heavy chain (hc) is followed by a flexible peptide linker to join it to the light chain (lc). The yellow sequences, corresponding to CDR1, CDR2 and CDR3, are shown in their approximate location within the heavy and light chains of the 4D5 antibody. Below the antibody structure is listed sequences corresponding to the CDR3 region of heavy and light chains. These sequences are replaced by the CDR3 sequences from the MEC1 surface IgG.

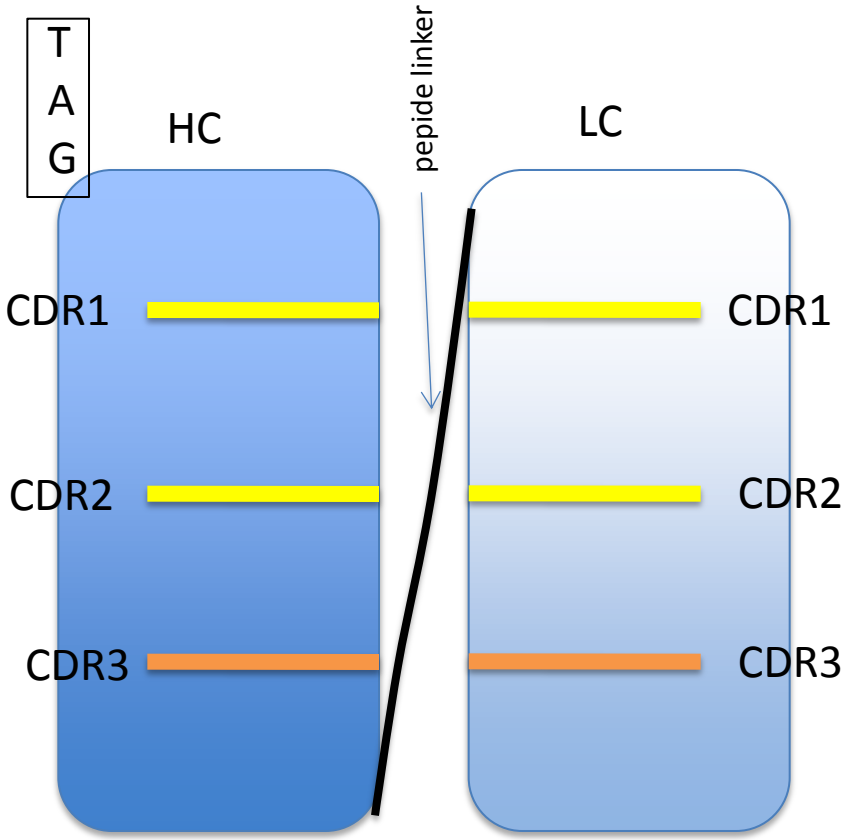
Fig 2

A. hum4D5 scFV - WT



H-CDR3 = SRWGGDGFYAMDY 13aa
L-CDR3 = QQHYTTPPT 9 aa

B. hum4D5 scFV – JVM-13_CDR3s

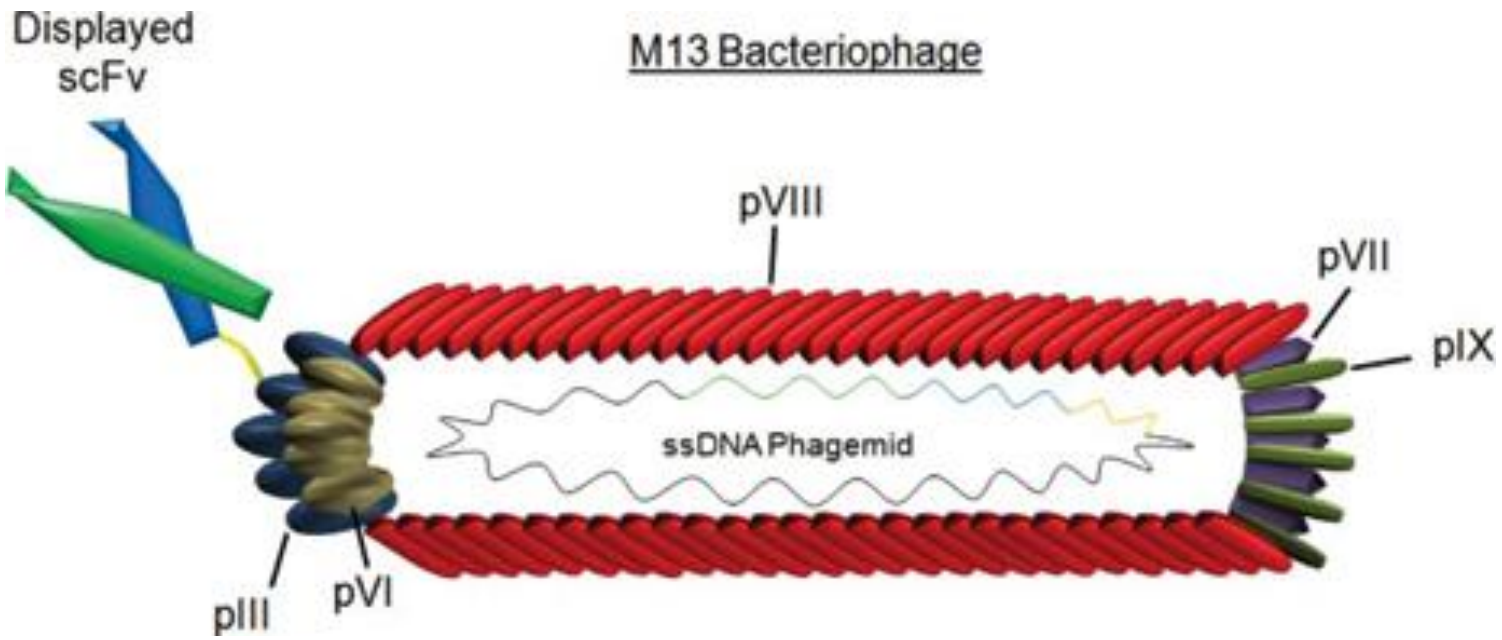


H-CDR3 = ASSYYDILTGYLY 13aa
L-CDR3 = SSYTSSSTLMI 11aa

Legend to Fig 2.

Generation of target proteins by inserting JVM13 CDR3 sequences into the scaffold antibody, 4D5. The idealized structure of the single chain Fv of the 4D5 antibody is shown on the left. The heavy chain (hc) is followed by a flexible peptide linker to join it to the light chain (lc). The yellow sequences, corresponding to CDR1, CDR2 and CDR3, are shown in their approximate location within the heavy and light chains of the 4D5 antibody. Below the antibody structure is listed sequences corresponding to the CDR3 region of heavy and light chains. These sequences are replaced by the CDR3 sequences from the JVM13 surface IgG.

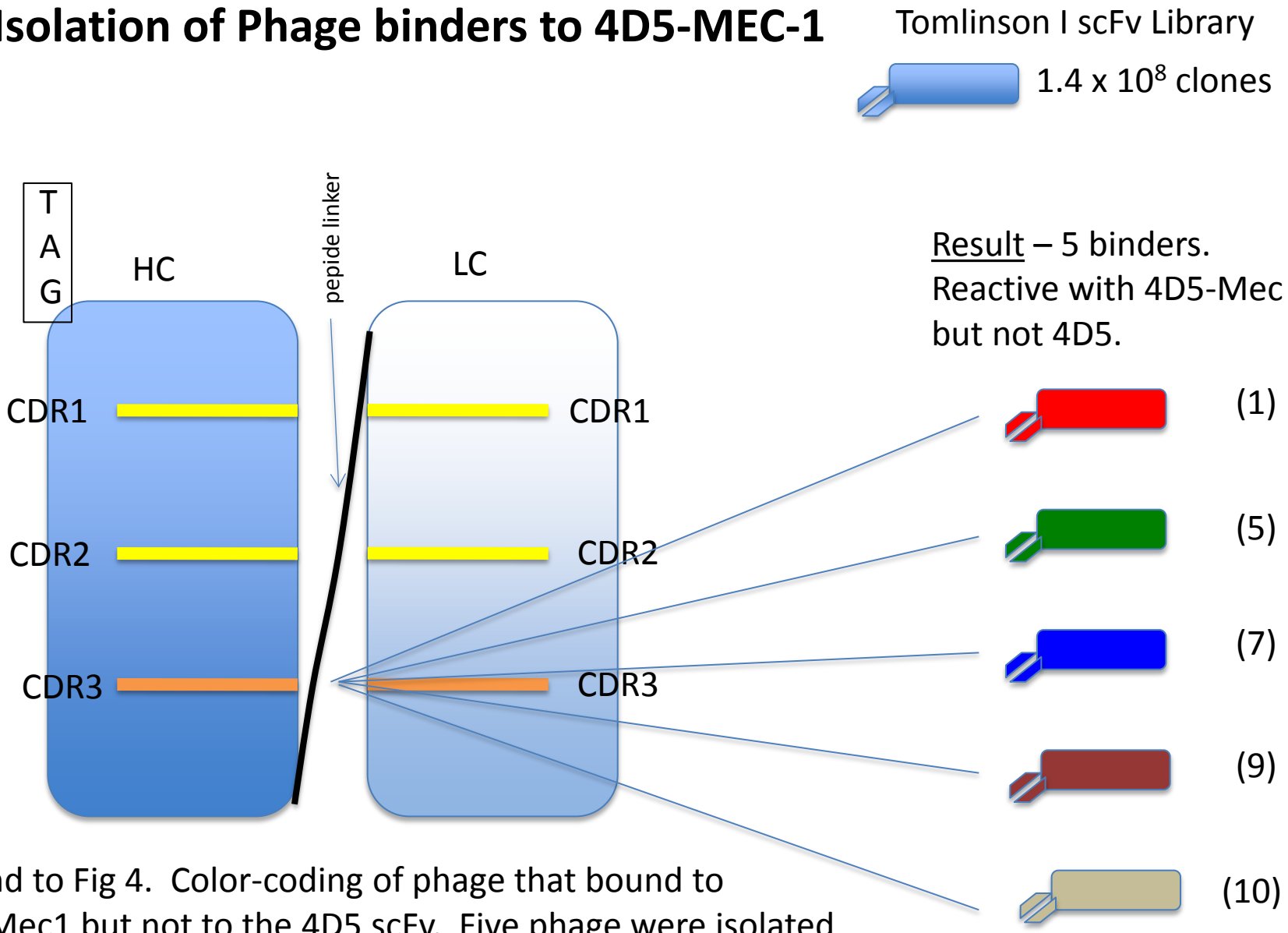
Fig 3.



Legend to Fig 3. Shown is the M13 bacteriophage displaying a single chain Fv fused with one copy of the pIII protein.

Fig 4.

Isolation of Phage binders to 4D5-MEC-1



Legend to Fig 4. Color-coding of phage that bound to 4D5-Mec1 but not to the 4D5 scFv. Five phage were isolated with unique sequences. The sequence of each phage is provide in Figures 5, 6, 7, 8 and 9.

Fig 5.

Isolate #1 DNA/and corresponding protein

atggccgaggtgcagctgttggaatctgggggaggcttggtacagcctgggggtccctg
M A E V Q L L E S G G G L V Q P G G S L
agactctcctgtgcagcctctggattcacctttagcagctatgccatgagctgggtccgc
R L S C A A S G F T F S S Y A M S W V R
caggctccagggaaggggctggagtgggtctcatatattacttctaatggtaataataca
Q A P G K G L E W V S Y I T S N G N N T
tcttacgcagactccgtgaagggccggttcaccatctccagagacaattccaagaacacg
S Y A D S V K G R F T I S R D N S K N T
ctgtatctgcaaatgaacagcctgagagccgaggacacggccgtatattactgtgcgaaa
L Y L Q M N S L R A E D T A V Y Y C A K
actggtggttcttttgactactggggccagggaaccctggtcaccgtctcgagcggtgga
T G G S F D Y W G Q G T L V T V S S G G
ggcggttcaggcggaggtggcagcggcggtggcgggtcgacggacatccagatgacccag
G G S G G G G S G G G G S T D I Q M T Q
tctccatcctccctgtctgcatctgtaggagacagagtcaccatcacttgccgggcaagt
S P S S L S A S V G D R V T I T C R A S
cagagcattagcagctatttaaattggtatcagcagaaaccagggaaagcccctaagctc
Q S I S S Y L N W Y Q Q K P G K A P K L
ctgatctataatgcatccaatttgcaaagtggggtcccatcaaggttcagtggcagtgga
L I Y N A S N L Q S G V P S R F S G S G
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S G T D F T L T I S S L Q P E D F A T Y
tactgtcaacagtctgatagtaatcctgatacgttcggccaagggaccaaggtggaaatc
Y C Q Q S D S N P D T F G Q G T K V E I
aaa
K

MAEVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSYIT
SNGNNT
SYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKTGGSFDYWGQGTLVT
VSSGG
GGSGGGGSGGGGSTDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKP
GKAPKL
LIYNASNLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSDSNPDTFGQGT

Fig 6. Isolate #5 DNA/and corresponding protein

atggccgaggtgcagctgttggagtctgggggaggcttggtatagcctgggggtccctg
M A E V Q L L E S G G G L V - P G G S L
agactctcctgtgcagcctctggattcacctttagcagctatgccatgagctgggtccgc
R L S C A A S G F T F S S Y A M S W V R
caggctccagggaaggggctggagtgggtctcagctattaatagtaatggtgcttataca
Q A P G K G L E W V S A I N S N G A Y T
tcttacgcagactccgtgaagggccggttcaccatctccagagacaattccaagaacacg
S Y A D S V K G R F T I S R D N S K N T
ctgtatctgcaaatgaacagcctgagagccgaggacacggccgtatattactgtgcgaaa
L Y L Q M N S L R A E D T A V Y Y C A K
agttctggttcttttgactactggggccagggaaccctggtcaccgtctcgagcggtgga
S S G S F D Y W G Q G T L V T V S S G G
ggcggttcaggcggaggtggcagcggcggtggcgggtcgacggacatccagatgacccag
G G S G G G G S G G G G S T D I Q M T Q
tctccatcctccctgtctgcatctgtaggagacagagtcaccatcacttgccgggcaagt
S P S S L S A S V G D R V T I T C R A S
cagagcattagcagctatttaaattggtatcagcagaaaccagggaaagcccctaagcac
Q S I S S Y L N W Y Q Q K P G K A P K H
ctgatctatgatgcatccggtttgcaaagtggggtcccatcaaggttcagtggcagtgga
L I Y D A S G L Q S G V P S R F S G S G
tctgggacagatttcactctcaccatcagcagtctgcaacctgaagattttgcaacttac
S G T D F T L T I S S L Q P E D F A T Y
tactgtcaacagtctggtgctactcctggtacgttcggccaagggaccaaggtggaaatc
Y C Q Q S G A T P G T F G Q G T K V E I
aaa
K

TAG suppressor

MAEVQLLESGGGLV-
PGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAINSNGAYT
SYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKSSGSFDYWGQGTLVTVSSG
G
GGSGGGGSGGGGSTDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAP
KH
LIYDASGLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSGATPGTFGQGTKVEI
K

Fig 7. Isolate #7 DNA/and corresponding protein

atggccgaggtgcagctgttggagtctgggggaggcttggtacagcctgggggtccctg
M A E V Q L L E S G G G L V Q P G G S L
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R L S C A A S G F T F S S Y A M S W V R
caggctccagggaaggggctggagtgggtctcaaatattactgatagtggttatactaca
Q A P G K G L E W V S N I T D S G Y T T
tcttacgcagactccgtgaagggcaggttcaccatctccagagacaattccaagaacacg
S Y A D S V K G R F T I S R D N S K N T
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L Y L Q M N S L R A E D T A V Y Y C A K
actggtaatagttttgactactggggccagggaaccctggtgaccgtctcgagcggtgga
T G N S F D Y W G Q G T L V T V S S G G
ggcggttcaggcggaggtggcagcggcggtggcgggtcgacggacatccagatgacccag
G G S G G G G S G G G G S T D I Q M T Q
tctccatcctccctgtctgcatctgtaggagacagagtcaccatcacttgccgggcaagt
S P S S L S A S V G D R V T I T C R A S
cagagcattagcagctatttaaattggtatcagcagaaaccagggaaagcccctaagcac
Q S I S S Y L N W Y Q Q K P G K A P K H
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L I Y A A S S Y L Q S G V P S R F S G S G
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S G T D F T L T I S S L Q P E D F A T Y
tactgtcaacatgatactactactcctagtacgttcggccaagggaccaaggtggaaatc
Y C Q H D T T T P S T F G Q G T K V E I
aaa
K

MAEVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSNITDSGY
TT
SYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKTGNSFDYWGQGTLVTVSSG
G
GGSGGGGSGGGGSTDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAP
KH
LIYAASYLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQHDTTTPSTFGQGTKVEI
K

Fig 8.

Isolate #9 DNA/and corresponding protein

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M A E V Q L L E S G G G L V Q P G G S L
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R L S C A A S G F T F S S Y A M S W V R
caggctccagggaaggggctggagtgggtctcaaatattacttctagtggtactggtaca
Q A P G K G L E W V S N I T S S G T G T
tattacgcagactccgtgaagggcaggttcaccatctccagagacaattccaagaacacg
Y Y A D S V K G R F T I S R D N S K N T
ctgtatctgcaaatgaacagcctgagagccgaggacacggccgtatattactgtgcgaaa
L Y L Q M N S L R A E D T A V Y Y C A K
gcttcttctgcttttgactactggggccagggaaccctggtcaccgtctcgagcggtgga
A S S A F D Y W G Q G T L V T V S S G G
ggcggttcaggcggaggtggcagcggcggtggcgggtcgacggacatccagatgacccag
G G S G G G G S G G G G S T D I Q M T Q
tctccatcctccctgtctgcatctgtaggagacagagtcaccatcacttgccgggcaagt
S P S S L S A S V G D R V T I T C R A S
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Q S I S S Y L N W Y Q Q K P G K A P K L
ctgatctataatgcatccgatttgcaaagtggggtcccatcaaggttcagtggcagtgga
L I Y N A S D L Q S G V P S R F S G S G
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S G T D F T L T I S S L Q P E D F A T Y
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Y C Q Q D N S T P D T F G Q G T K V E I
aaa
K

MAEVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSNITSSGTGT
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GGSGGGGSGGGGSTDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKL
LIYNASDLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQDNSTPDTFGQGTKVEI
K

Fig 9

Isolate #10 DNA/and corresponding protein

TAG suppressor

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M A E V Q L L E S G G G L V Q P G G S L
agactctcctgtgcagcctctggattcacctttagcagctatgccatgagctgggtccgc
R L S C A A S G F T F S S Y A M S W V R
caggctccagggaaggggctggagtaggtctcatatattgctgctgagggtagtactaca
Q A P G K G L E - V S Y I A A E G S T T
tattacgcagactccgtgaagggccggttcaccatctccagagacaattccaagaacacg
Y Y A D S V K G R F T I S R D N S K N T
ctgtatctgcaaatgaacagcctgagagccgaggacacggccgtatattactgtgcgaaa
L Y L Q M N S L R A E D T A V Y Y C A K
tctactggtacttttgactactggggccagggaaccctggtcaccgtctcgagcggtgga
S T G T F D Y W G Q G T L V T V S S G G
ggcggttcaggcggaggtggcagcggcggtggcgggtcgacggacatccagatgacccag
G G S G G G G S G G G G S T D I Q M T Q
tctccatcctccctgtctgcatctgtaggagacagagtcaccatcacttgccgggcaagt
S P S S L S A S V G D R V T I T C R A S
cagagcattagcagctatttaaattggtatcagcagaaaccagggaaagcccctaagctc
Q S I S S Y L N W Y Q Q K P G K A P K L
ctgatctataatgcatccgctttgcaaagtggggtcccatcaaggttcagtggcagtgga
L I Y N A S A L Q S G V P S R F S G S G
tctgggacagatttcactctcaccatcagcagtctgcaacctgaagattttgcaacttac
S G T D F T L T I S S L Q P E D F A T Y
tactgtcaacaggatgattctgctcctgatacgttcggccaagggaccaaggtggaaatc
Y C Q Q D D S A P D T F G Q G T K V E I
aaa
K

MAEVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLE-VSYIAAEGSTT
YYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKSTGTFDYWGQGTLVTVSSGG
GGSGGGGSGGGGSTDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKL
LIYNASALQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQDDSAPDTFGQGTKVEI
K

Fig 10 Alignments confirms that the antibody Fvs from each phage represents a unique sequence

```
Isolate1     1 MAEVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSYITSNGNNT  60
Isolate5     1 MAEVQLLESGGGLVXPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAINSNGAYT  60
Isolate7     1 MAEVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSNITDSGYTT  60
Isolate9     1 MAEVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSNITSSGTGT  60
Isolate10    1 MAEVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEXVSYIAAEGSTT  60
           ************** ********************************** ** *   *  *

Isolate1    61 SYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKTGGSFDYWGQGTLVTVSSGG 120
Isolate5    61 SYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKSSGSFDYWGQGTLVTVSSGG 120
Isolate7    61 SYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKTGNSFDYWGQGTLVTVSSGG 120
Isolate9    61 YYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKASSAFDYWGQGTLVTVSSGG 120
Isolate10   61 YYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKSTGTFDYWGQGTLVTVSSGG 120
           ************************************    ****************

Isolate1   121 GGSGGGGSGGGGSTDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKL 180
Isolate5   121 GGSGGGGSGGGGSTDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKH 180
Isolate7   121 GGSGGGGSGGGGSTDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKH 180
Isolate9   121 GGSGGGGSGGGGSTDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKL 180
Isolate10  121 GGSGGGGSGGGGSTDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKL 180
           ************************************************************

Isolate1   181 LIYNASNLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSDSNPDTFGQGTKVEI 240
Isolate5   181 LIYDASGLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSGATPGTFGQGTKVEI 240
Isolate7   181 LIYAASYLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQHDTTTPSTFGQGTKVEI 240
Isolate9   181 LIYNASDLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQDNSTPDTFGQGTKVEI 240
Isolate10  181 LIYNASALQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQDDSAPDTFGQGTKVEI 240
           *** ** ************************************     * **********

Isolate1   241 K 241
Isolate5   241 K 241
Isolate7   241 K 241
Isolate9   241 K 241
Isolate10  241 K 241
           *
```

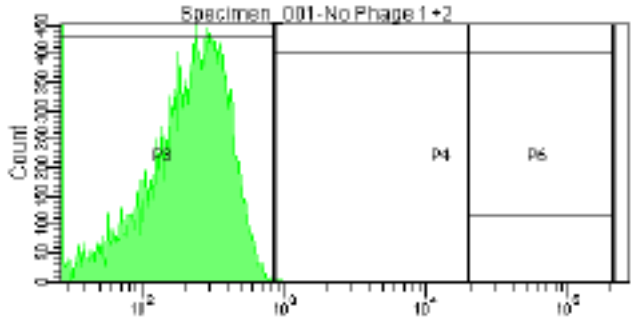
Legend to FIg 11. X equals location of stop codon. Stop codons were engineered/mutated to encode either the normal framework residue Q and W respectively or replaced with 'E' – the amino acid inserted by the suppressor strain.

Fig 11.

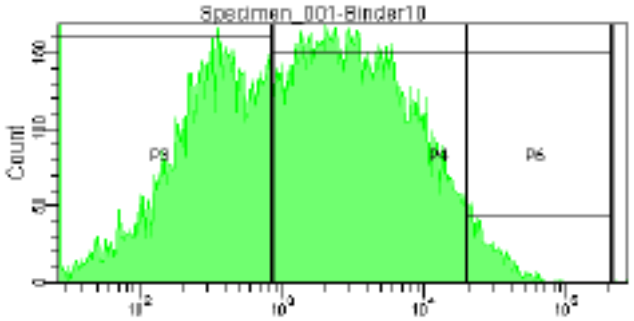
Phage binding to the surface of MEC-1 Cells

Phage #

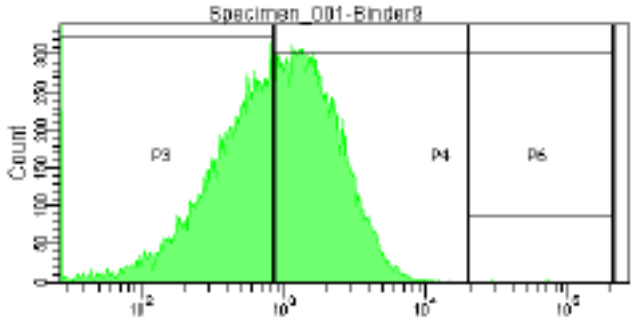
(-)



(10)

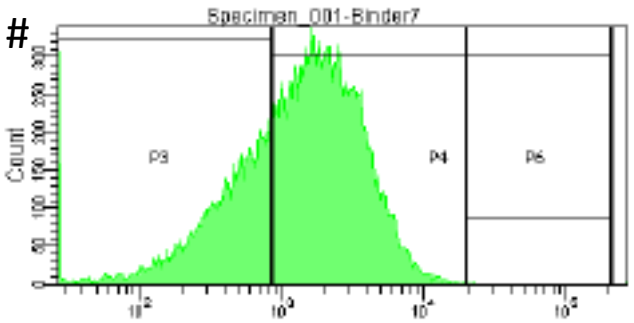


(9)

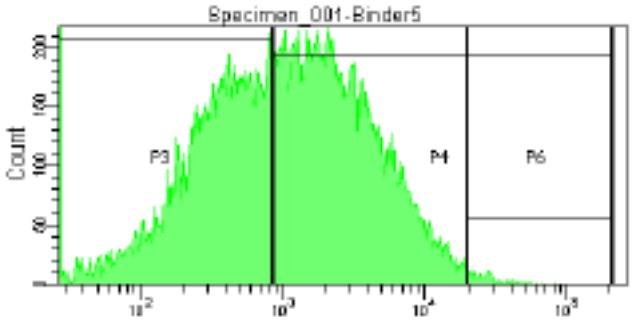


Phage #

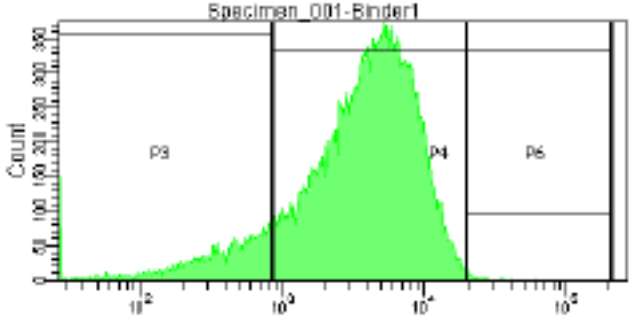
(7)



(5)



(1)



Legend to Fig 12. Data shows binding in area 'P4' of phage to intact Mec-1 cells.